

CLAIMS

1. A method for intermediate to large scale production in a semi-solid culture medium of stock compositions of bacteriophage having a titer of at least 10^{11} pfu/ml and a total yield of at least 10^{15} total pfu comprising:
 - 5 a. growing bacteriophage in a semi-solid culture medium comprising a pre-incubated mixture of at least one bacterial strain and at least one phage type further comprising hydrocolloid at a concentration below 0.5%;
 - b. incubating the semi-solid culture medium to reach bacterial lysis, thereby obtaining a phage lysate; and
 - 10 c. extracting a crude bacteriophage extract from the semi-solid culture medium, using an extraction medium.
2. The method according to claim 1, wherein the volume of the semi-solid culture medium is in the range of 1-20 liters.
3. The method according to claim 1, wherein the volume of the extraction medium is in the range of 20 to 100 fold the volume of the semi-solid culture medium.
- 15 4. The method according to claim 1, wherein the semi-solid culture medium comprises a hydrocolloid at a concentration below 0.3%.
5. The method according to claim 1, wherein the semi-solid culture medium comprises a hydrocolloid at a concentration of 0.25%-0.30%.
- 20 6. The method according to claim 1, wherein the hydrocolloid is selected from the group consisting of agar, agarose, starch, pectin, carrageenan, alginate, gelatin, gellan, konjak mannan, xanthan and gum, and combinations thereof.
7. The method according to claim 6, wherein the hydrocolloid is agar.
- 25 8. The method according to claim 1, wherein the pre-incubated mixture comprises bacteria and bacteriophage at a ratio of from about 10^8 to about 10^9 colony forming units to one bacteriophage plaque, further comprises a rich medium.

9. The method according to claim 1, wherein the crude bacteriophage extract is obtained by sequential serial extractions.
10. The method according to claim 1, wherein the semi-solid culture medium is supported by a solid phase.
- 5 11. The method according to claim 10, wherein the semi-solid culture medium is layered on top of a first supportive solid phase bottom layer to form a second top layer.
12. The method according to any one of claims 10-11 wherein the solid phase comprises a hydrocolloid at a concentration range of 1.0-2.0%.
- 10 13. The method according to claim 12 wherein the solid phase comprises agar at a concentration range of 1.0-2.0%.
14. The method according to any one of claims 10-13 wherein the volume of the solid phase is from about two to about ten fold the total volume of the semi-solid culture medium that it is intended to support.
15. The method according to claim 1 wherein the titer of the crude bacteriophage extract is at least 10^{11} pfu/ml.
16. The method according to claim 1 wherein the titer of the crude bacteriophage extract is in a range of 5×10^{11} to 10^{12} pfu/ml.
17. The method according to claim 1 wherein the bacteriophage yield is at least 20 is the order of magnitude of 10^{15} to 10^{16} total pfu.
18. The method according to claim 1 further comprising purifying the crude bacteriophage extract to obtain a bacteriophage stock composition by a method selected from the group consisting of fractionation by PEG, CsCl gradient centrifugation, filtration, ultra-filtration, and column chromatography.
- 25 19. The method according to claim 18 wherein the purified bacteriophage stock composition is lyophilized.

20. A purified, specific bacteriophage composition for use in phage therapy comprising a bacteriophage stock composition wherein the bacteriophage stock composition is produced in a semi-solid culture medium, said composition having a titer of at least 10^{11} pfu/ml and a total yield of at least 5 10^{15} total pfu by a method comprising:
- growing bacteriophage in a semi-solid culture medium comprising a pre-incubated mixture of at least one bacterial strain and at least one phage type further comprising hydrocolloid at a concentration below 0.5%;
 - incubating the semi-solid culture medium to reach bacterial lysis, thereby obtaining a phage lysate;
 - extracting a crude bacteriophage extract from the semi-solid culture medium using an extraction medium; and
 - purifying the crude bacteriophage extract.
- 10 21. The composition according to claim 20, wherein the volume of the semi-solid culture medium is in the range of 1-20 liters.
- 15 22. The composition according to claim 20 wherein the volume of the extraction medium is in the range of 20 to 100 fold the volume of the semi-solid culture medium.
- 20 23. The composition according to claim 20 wherein the crude bacteriophage extract is extracted from a semi-solid culture medium comprising hydrocolloid at a concentration below 0.3%.
24. The composition according to claim 20 wherein the crude bacteriophage extract is extracted from a semi-solid culture medium comprising hydrocolloid at a concentration of 0.25%-0.30%.
25. The composition according to claim 20 wherein the crude bacteriophage extract is extracted from a semi-solid culture medium comprising hydrocolloid selected from the group consisting of agar, agarose, starch, pectin, carrageenan, alginate, gelatin, gellan, konjak mannan, xanthan and gum, and combinations thereof.

26. The composition according to claim 25 wherein the hydrocolloid is agar.
27. The composition according to claim 20 wherein the crude bacteriophage extract is obtained by sequential serial extractions.
28. The composition according to claim 20 wherein the pre-incubated mixture comprises bacteria and bacteriophage at a ratio of from about 10^8 to about 10^9 colony forming units to one bacteriophage plaque, further comprises a rich medium.
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29. The composition according to claim 20 wherein the semi-solid culture medium is supported by a solid phase.
30. The composition according to claim 29 wherein the semi-solid culture medium is layered on top of a first supportive solid phase bottom layer to form a second top layer.
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31. The composition according to claim 30 wherein the supportive solid phase comprises hydrocolloid at a concentration range of 1.0-2.0%.
32. The composition according to claim 31 wherein the supportive solid phase comprises agar at a concentration range of 1.0-2.0%.
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33. The composition according to claim 29 wherein the volume of the solid phase is from about two to about ten fold the total volume of the semi-solid culture medium that it is intended to support.
34. The composition according to claim 20 wherein the purification of the crude bacteriophage extract is performed by a method selected from the group consisting of fractionation by PEG, CsCl gradient centrifugation, filtration, ultra-filtration, and column chromatography.
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35. The composition according to claim 34 wherein the purified bacteriophage composition is at a titer of at least 10^{11} pfu/ml.
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36. The composition according to claim 35 wherein the titer of the purified bacteriophage composition is at a range of $5 \times 10^{11} - 10^{12}$ pfu/ml.

37. The composition according to claim 36 wherein the purified bacteriophage composition is lyophilized.
38. The composition according to claim 20 further comprising at least one sugar that reduces or abolishes bacterial phage-neutralizing activity.
- 5 39. The composition according to claim 38 wherein the sugar is selected from the group consisting of N-acetyl-D-glucosamine, 2-deoxy-D-glucose, maltose, L-rhamnose, cellobiose, and D-xylose.
40. The composition according to claim 39 wherein the sugar is present in the stock composition at a concentration in the range of 0.2-2.0 M.
- 10 41. The composition according to claim 38 wherein the purified bacteriophage stock composition comprises *Pseudomonas aeruginosa* phage and the sugar is selected from the group consisting of D-glucosamine, D-mannose and L-rhamnose.
42. The composition according to claim 41 wherein the inhibitory sugar is at a concentration in the range of 0.02-0.5 M.
- 15 43. The composition according to claim 20 further comprising a pharmaceutically acceptable diluent or carrier.